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Well-defined carbohydrate-based polymers in calcium carbonate crystallization: Influence of stereochemistry in the polymer side chain on polymorphism and morphology

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ABSTRACT

In this work we demonstrate the remarkable phase control on the crystallization of calcium carbonate by the stereochemistry of carbohydrate-based polymers. The polymers (poly(2-(2,3,4,6-tetra-O-acetyl-β-D-glucosyloxy)ethyl methacrylate) and poly(2-(2,3,4,6-tetra-O-acetyl-β-D-galactosyloxy)ethyl methacrylate)) have been synthesized from the respective glucose or galactose containing monomers (3 step synthesis) by RAFT polymerization leading to well-defined carbohydrate-based polymers with number averages of the molecular weights (M_w) of 10,000–18,000 g/mol and a dispersities (Đ) from 1.1 to 1.2. For the deprotected polymers we found differences in the phase selection of calcium carbonate. We found that this effect is based on the chelating character of the hydroxyl groups of the pyranoses and their individual orientation, as demonstrated by comparison of the protected and unprotected polymers in crystallization experiments as well as computer assisted simulations.

1. Introduction

Biomineralization is a common phenomenon in nature leading to the formation of a variety of solid inorganic structures by living organisms. In this category we classify materials such as intracellular crystals in prokaryotes, exoskeletons in protozoa, algae, and invertebrates, spicules and lenses, bone, teeth, statoliths, and otoliths, eggshells, plant mineral structures, and also pathological biominerals such as gall stones, kidney stones, and oyster pearls [1–5], which are formed under biological control. From a chemical point of view, they are inorganic–organic hybrid composites [6] formed by self-assembly bottom up processes under ambient conditions, exhibiting interesting properties, controlled hierarchical structures, and remodeling [7–9]. Therefore, the biomimetic formation of biominerals provides a unique approach for controlling calcium carbonate formation [10,11] and for the design of materials in general [12–15].

Up to now a large number of proteins have been identified that are involved in the control of biomineralization processes [16–18]. Many of them are negatively charged and contain carboxylate, sulfate, or phosphate functional groups, which may bind Ca^{2+} ions thereby controlling nucleation and crystal growth by lowering the interfacial energy between the crystal and the macromolecular substrate [19–25]. On the other hand since Abolins-Krogis'

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work [26], a slow but increasing interest has developed to explore the role of saccharides, polysaccharides and proteoglycans in biomineralization [27–30]. This is due to the fact that polysaccharides appear very early in the evolution of biomineralization [31]. Recently Zhong et al. [32] demonstrated that acidic polysaccharides can stabilize amorphous calcium carbonate. There is no single type of polysaccharide associated with biominerals, but such polysaccharides are typically hydroxylated, carboxylated or sulfonated, or they contain a mixture of these functionalities. Jäger et al. could show that the organic–mineral interface in bones is mostly based on polysaccharides [33,34]. The molecular interaction between calcium phosphate and polysaccharides seems essential for strength, adaptation and growth of bone. A detailed knowledge of these interactions is the key for an understanding and for a therapy of various bone diseases. The crystal formation of calcium phosphate or calcium carbonate has been reported to be strongly influenced by glycosides [35].

Therefore we have investigated in this work the influence of the stereochemistry of carbohydrate-based polymers on the crystallization of calcium carbonate. We synthesized two classes of carbohydrate-based polymers from galactosyloxyethyl methacrylate and glucosyloxyethyl methacrylate by controlled radical polymerization (RAFT) [36–38] to obtain well defined carbohydrate-based polymers [39,40]. The polymeric structures offer a multivalent binding to a crystal surface comparable to that of higher polysaccharides, but they also offer a much better accessibility. As we were interested in the effect of stereochemistry on phase selection and the crystal morphology we synthesized polymers with different sugar side groups, but nearly identical molecular weight. The monomers, which are galactose and glucose based, differ in the stereochemistry of the hydroxyl group in position 4 of the pyranose form. In the case of galactose the hydroxyl group is in axial, for glucose in equatorial position. In all other aspects the monomers and polymers are identical. To study the influence of these polymers on calcium carbonate crystallization we have carried out crystallizations following the standard ammonium carbonate method.

2. Experimental part

2.1. Materials

All chemicals were obtained either from Aldrich or ACROS and are reagent grade. The chemicals were used without further purification unless indicated otherwise. Dioxane used in the synthesis was freshly distilled from a sodium/potassium mixture. 2,2′-Azobis(isobutyronitrile) (AIBN) was recrystallized from diethyl ether and stored at −7 °C. 2-Hydroxyethyl methacrylate (HEMA) was freshly distilled before reaction.

2.2. Characterization

^{1}H NMR spectra were obtained at 300 MHz using a FT-spectrometer from Bruker and analyzed using the ACDLabs 6.0 software. The polymers were dried at 40 °C over night under vacuum and submitted afterward to gel permeation chromatography (GPC). GPC was performed in tetrahydrofuran (THF) as solvent using the following set up: pump PU 1580, auto sampler AS 1555, UV-detector UV 1575, RI-detector RI 1530 from Jasco and miniDAWN Tristar light scattering detector from Wyatt. Columns were used from MZ-Analysentechnik: MZ-Gel SDplus 10^2 Å, MZ-Gel SDplus 10^4 Å and MZ-Gel SDplus 10^6 Å. The elution diagrams were analyzed using the ASTRA 4.73.04 software from Wyatt Technology. Calibration was done using polystyrene standards. The flow rate was 1 mL/min at a temperature of 25 °C.

2.3. Glycopolymer syntheses

2.3.1. Synthesis of the chain transfer agent (CTA)

The 4-cyano-4-((thiobenzoyl) sulfanyl)pentanoic acid was used as the chain transfer agent (CTA) and synthesized according to the literature [41].

2.3.2. Synthesis of 1,2,3,4,6-penta-O-acetyl-α-D-glucopyranose (**1**)

The 1,2,3,4,6-penta-O-acetyl-α-D-glucopyranose was synthesized according to the standard procedure [42]. 472 mL of acetanhydrid and 472 mL of pyridine were cooled to 0 °C in an ice bath. 100 g (0.56 mol) of α-D-glucose was added slowly to the solution in order to keep the temperature of the mixture below 10 °C. After the glucose was completely dissolved, the solution was stirred at 0 °C for 1 h and later 16 h at room temperature. The conversion was observed by TLC [R_f: 0.51 (3:2/toluene:ethyl acetate)]. After full conversion the solution was cooled again to 0 °C and hydrolyzed slowly by addition of 850 mL of water. The mixture was extracted 3 times using 500 mL of chloroform each time. The extracted organic phase was washed three times with 500 mL 1 N hydrochloride solution and one time with saturated sodium chloride solution. The organic phase was dried using magnesium sulfate, and the remaining solvent was removed. The residue was codestilled 3 times with toluene. Yield: 199.9 g (0.56 mol, quant.), slightly yellowish solid.

2.3.3. Synthesis of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (**2**)

The 1,2,3,4,6-penta-O-acetyl-α-D-glucopyranosyl bromide was synthesized according to the standard procedure [43]. A solution of 20.0 g (51.2 mmol) 2,3,4,6-tetra-O-acetyl-α-D-glucopyranose (**1**) in 500 mL dichloromethane was cooled to 0 °C. Over 50 min 113 mL of a 33% solution of hydrobromic acid in glacial acetic acid was added. The conversion was monitored by TLC [R_f: 0.58 (1:1/cyclohexane:ethylacetate)]. After 60 min 125 mL of ice water were added. The organic phase was washed 3 times with 200 mL of saturated sodium bicarbonate solution and two times with 200 mL of saturated sodium thiosulfate. The organic phase was dried using magnesium sulfate, and the remaining solvent was removed. The crude product was purified by flash chromatography (solvents: cyclohexane/ethylacetate, 3:1). Yield: 14.6 g (35.5 mmol, 69%), colorless amorphous solid, $C_{14}H_{19}BrO_9$ (411.20 g mol^{-1}) ESI-MS (positive), m/z (%): 353.13 (100%) ([$C_{14}H_{18}O_9$ + Na]$^+$,

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